expressing phage, a synthetic gene for activated 65- kDa Cry1Ac toxin (patterned after the *Bt* subspecies kurstaki *cry1Ac* sequence, codon-optimized for high expression in plants (37), GenBank Accession number U63372), was amplified from plasmid pAGM19 with the primers LK01(5'-GTG AGT GAG T*GG CCG ACG GGG CC*G CTG GAA TGG ACA ACA ATC CCA ACA TC-3') (SEQ ID NO. 1) and LK02 (5' -TGA GTG AGT C*GG CCC CAG AGG CC*C TGC AGC TCC CTC GAG CGT TGC AGT AAC GGG-3') (SEQ ID NO. 2). These primers amplified the *cry1Ac* sequence (codons 1-616) and added *Sfi*I sites to each end (*cry1Ac* homologous sequence in the primers is underlined, *Sfi*I sites are italic). The 1.8-kb PCR product was digested with *Sfi*I and ligated to the 9.6-kb *Sfi*I digested fUSE5 vector, transformed into JM109 cells, and selected for growth on tetracycline media. Phage produced by fUSE5 do not kill their host cells and so grow as colonies on selective agar (35). Twenty colonies were selected at random and inoculated into 3 ml LB-tetracycline liquid cultures. The supernatants of these overnight cultures were screened for the presence of transducing units (tu) indicating production of functional phage. All of the supernatants were positive and contained approximately equal titers of phage. The cell pellets of the overnight cultures were processed for plasmid purification and the resulting DNA subjected to restriction analysis. The analysis revealed that 5 of the 20 colonies contained inserts of the appropriate 1.8-kb size, one had a slightly shorter insert, and the rest contained no insert. The five phage isolates carrying complete putative *cry1Ac* genes and a no insert fUSE5 control were purified from 50 ml cultures. Each was then individually combined with an artificial insect diet and fed to tobacco budworms (*Heliothis virescens)*, a Cry1Ac-susceptible insect, in a simple single-dose feeding assay. The isolate found to be most toxic to the larvae (hereafter referred to as the 1Ac-fUSE5 phage) was sequenced through the *cry1Ac* and *cpIII* junction regions (Sequenase, USB) and kept for further experiments.

D2

To create the 1Ac-Kpn-fUSE5 phage, which contains a unique KpnI site at the junction of domains I and II of Cry1Ac, a G->C mutation was introduced into codon 279 by PCR mutagenesis as follows. The Cry1Ac gene in pAGM19 was amplified in two parts. Primers LK01 (above) and LK04 (5'-GA GCC TCG AAA GGT ACC GTC-3') (SEQ ID NO. 3) in one reaction amplified the 5' end of the gene, codons 1 to 283. Primers LK02 (above) and LK03 (5'-GAC GGT ACC TTT CGA GGC TC-3') (SEQ ID NO. 4) in a

nucleotide in primers LK03 and LK04 is underlined. The whole gene was then reassembled in a third 100 ul PCR reaction containing 1 ul of each of the preceding two reaction products, and 10 pMoles each of primers LK01 and LK02. This 1.8-kb product was digested with *Sfi*I and cloned into the *Sfi*I sites of fUSE5. The entire sequence of the modified *cry1Ac* gene and the fusion junction with phage gene *pIII* was verified by DNA sequencing. This phage is referred to as 1Ac-Kpn-fUSE5 throughout this report.

Phage were purified by polyethylene glycol (PEG) precipitation (0.15 volumes of 16% [w/v] PEG 8000, 3.3 M NaCl) for 15 min on ice followed by centrifugation, and sometimes further re-precipitated with acetic acid (34).

SurfZap system The Stratagene Lambda SurfZAP™ vector is a 41.5-kb lambda phage vector derived from the LambdaZAP II™ vector (also Stratagene), which contains a defective filamentous phage (f1) genome that can be excised as a phagemid (pSurfscript) and packaged into f1 phage particles with the assistance of VCSM13 helper phage (17). A translational fusion of a *cry1Ac* gene with amino acids 198-406 of an f1 phage *cpIII* gene in the SurfZAP™ vector allows phage display of Cry1Ac protein on filamentous phage, and was constructed as follows. Codons 1 through 656 of a natural *B. thuringiensis cry1Ac* gene were PCR amplified from the OSU4202 construct (12) with primers that modified the ends as prescribed in the manufacturer's instructions. The upstream primer, PPELB (5' C T C G C T C G C C C A T A T / G C G G C C G C / A G G T C TCCTCCTCTTAGCAGCACAACCAGCAATGGCC/ATGGATAACAATCCGAACATCAT GAATGC-3') (SEQ ID NO. 5), provides a *NotI* site for ligation to the left lambda arm of SurfZAP, the remaining sequence to complete the *pelB* leader peptide (13 amino acids) and 30 nucleotides of homology to amino acids 1-10 of the *cry1Ac* coding region in frame with the *pelB* leader region (each segment delineated by "/"). The downstream primer, PCRY1 (5'-ATCCGATAAATA/GCTAG C/TAAATTGGACACTTGATCAATATGATAATCCG-3') (SEQ ID NO. 6), added an *NheI* site downstream of the *XhoI* site in *cry1Ac* which defines the C-terminal boundary of domain III of the active toxin and the protoxin coding region involved in crystal